

## Effects of rootstock on avocado fruit quality – assessment of postharvest disease, major cations and biochemical traits

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### Abstract

Growth and yield performance of 'Hass' and 'Shepard' grafted to several commercial and proprietary rootstocks has been assessed annually in field trials established around Australia between December 2004 and May 2005. Since 2008 fruit has been assessed for postharvest anthracnose and stem end rot after ripening at 23°C to favour disease development. Peel and/or flesh samples have been analysed for calcium and nitrogen, and for activities of peroxidase and catalase.

There were significant rootstock effects on disease and skin cations in some cases, for example, at Hampton in 2010, 'Hass' fruit from clonal SHSR-03, 'A10' and 'Velvick' had less anthracnose than from 'Reed' and 'Hass'. At the Walkamin field site in 2011 fruit skin calcium levels were highest in fruit from 'Shepard' trees on clonal 'Velvick' and seedling 'SHSR-03'. These rootstocks have typically performed well in terms of conferring resistance to anthracnose, thus re-affirming the importance of calcium in disease resistance.

### Introduction

In Australia and throughout the world, postharvest disease continues to be a major cause of avocado fruit quality loss in retail markets. Anthracnose (caused by the fungus *Colletotrichum gloeosporioides*) and stem end rot (caused by a range of fungi including *Botryosphaeria* spp.) are the two most important postharvest diseases of avocado in Australia (Pegg *et al.*, 2009). The fungi which cause these diseases initiate infection in the field and then remain in a quiescent state until after harvest when fruit commence ripening. For this reason, successful management of these diseases needs to include both field and postharvest strategies, such as fungicide application, orchard hygiene, nutritional optimisation, postharvest temperature and ripening management. The search for strategies which minimise fungicide use has provided the impetus for the research reported in this paper.

Field studies conducted over a number of years in Australia have demonstrated that rootstock can influence postharvest disease susceptibility in 'Hass' avocado. Willingham *et al.* (2001) reported that fruit anthracnose levels were significantly lower when 'Hass' was grafted to 'Velvick' rootstock as compared to 'Duke 6' rootstock. It was also shown that the 'Velvick/Hass' rootstock/scion combination had lower leaf N levels and higher leaf Ca+Mg/K ratios compared to the 'Duke 6/Hass' combination, suggesting a role for mineral nutrients in this rootstock-mediated disease resistance. Subsequent studies showed that 'Hass' trees grafted to the Guatemalan rootstocks 'Anderson 8' and 'Anderson 10' produced fruit with less anthracnose than those grafted to the Mexican race rootstock 'Parida 1' (Willingham *et al.*, 2006).

Given these encouraging early results, more extensive studies were conducted in conjunction with Dr Tony Whitley's national project on 'Rootstock Improvement for the Australian Avocado

Industry'. In this project, field trials were established at a number of sites across Australia between late 2004 and early 2005 to evaluate growth and yield performance of 'Hass' and 'Shepard' avocado trees grafted to a range of rootstocks. Over the last three years we have conducted postharvest quality assessments on fruit at four of these sites (Childers, Hampton and Walkamin in Queensland, and Pemberton in Western Australia). The assessments presented in this paper include postharvest disease (anthracnose and stem end rot following ripening at 23°C), fruit skin nutrient levels (eg. nitrogen and calcium) and fruit biochemicals potentially involved in disease resistance (eg. peroxidase and catalase) for 'Hass' and 'Shepard' fruit harvested during the 2010/11 avocado season.

## Materials and methods

During 2010/11, avocado fruit were harvested at commercial maturity for quality assessment from four trial sites across Australia as shown in Table 1.

Table 1: Commercial locations for collection of samples for fruit quality assessments.

Location	State	Date of harvest	Cultivar
Childers	QLD	19/5/10	Hass
Hampton	QLD	12/7/10	Hass
Pemberton	WA	1/12/10	Hass
Walkamin	QLD	7/2/11	Shepard

- At Childers, fruit from 'Hass' trees grafted to a selection of 11 rootstocks (six clonal and five seedling) were evaluated, with ten individual tree replications for each rootstock.
- At Hampton and Pemberton, fruit from 'Hass' trees grafted to a selection of six clonal rootstocks were evaluated at each site, with six individual tree replications for each rootstock.
- At Walkamin, fruit from 'Shepard' trees grafted to a selection of 11 rootstocks (six clonal and five seedling ones) were evaluated, with ten individual tree replications for each rootstock.

A list of the rootstocks selected at each site is shown in Table 2.

Table 2: Clonal (C) and seedling (S) rootstock selections with 'Hass' or 'Shepard' scion for fruit quality assessments at each of four harvests.

Childers (Hass)	Hampton (Hass)	Pemberton (Hass)	Walkamin (Shepard)
C <sup>1</sup> -A10	C-A10	C-A10	C-A10
C-Duke 7	C-Duke 7	C-Barr Duke	C-Duke 7
C-Nabal	C-Hass	C-Duke 7	C-Shepard
C- SHSR-03	C-Reed	C-Reed	C-SHSR-03
C-Velvick	C-SHSR-03	C-Velvick	C-Thomas
C-Zutano	C-Velvick	C-Zutano	C-Velvick
S <sup>2</sup> -A10			S-A10
S-Nabal			S-Duke 7
S-SHSR-02			S-SHSR-02
S- SHSR-03			S-SHSR-03
S-Velvick			S-Velvick

1 C=clonal; 2 S=seedling

Approximately 45 fruit/tree were harvested at each site, placed directly into trays and transported to the laboratory. Fruit arrived within 24 hours of picking for Childers and Hampton 'Hass' fruit, and within 48 hours of picking for Pemberton 'Hass' fruit. 'Shepard' fruit from Walkamin were consigned to Brisbane by refrigerated (approximately 7°C) road freight and arrived within 72 hours of harvest, with the exception of fruit to be sampled for nutrients and biochemical analysis which were consigned to Brisbane by air freight and arrived within 24 hours of harvest.

From each sample of 45 fruit/tree, 20 fruit were transferred to a ripening room at 23°C and 65% relative humidity (DEEDI Indooroopilly or Ecosciences Precinct) for assessment of postharvest disease. These conditions were used to maximise disease expression. At the eating ripe stage (as judged by fruit firmness), 'Hass' fruit were peeled and assessed for anthracnose and stem end rot. 'Shepard' fruit were assessed for anthracnose externally without peeling, and stem end rot was assessed by cutting through the peel and flesh to see the extent of infection. In each replication, the percentage of fruit with 5% or less anthracnose severity and no stem-end rot was considered marketable.

The remaining 25 fruit/tree were sent to the DEEDI laboratories at Nambour. Twenty fruit per tree were held at 5°C for four weeks, then ripened at 20°C until eating ripe (based on fruit firmness), with no ethylene treatment. These cold-stored fruit were assessed for skin colour, internal disorders and diseases at the eating ripe stage. Data from these assessments are not presented in this paper.

The remaining five fruit per tree were sampled for mineral biochemical analysis:

- Skin samples were oven-dried at 60°C to constant weight, ground and sent to a commercial laboratory (SGS Agritech, Toowoomba) to determine the concentrations of N and Ca (the minerals most commonly associated with fruit quality after harvest) using standard certified techniques.
- Two enzymes known to be involved in plant defence reactions, peroxidase and catalase, were assayed in the flesh of fruit sampled at harvest time. Samples were only taken from fruit on trees grafted to the clonal rootstocks (five replicate trees for each of the six clonal rootstocks). Small sections of avocado flesh were taken from each of five fruit per rootstock and pooled together. The sample was ground to a fine powder in liquid nitrogen, then in sodium phosphate buffer to prevent degradation of the enzymes. Cell debris was removed through centrifugation and the supernatant was used for the assays. The extract was diluted, a substrate specific to the enzyme was added, and the degradation of the substrate was measured over time to obtain the enzyme activity rate. Each assay was replicated five times and the average was taken. Protein levels were determined for each sample and the enzyme rate per milligram of protein calculated.

Statistical analyses (analysis of variance) were performed using Genstat 11 for Windows (VSN International Ltd., UK). The protected least significant difference (LSD) procedure at  $P = 0.05$  was used to test for differences between treatment means.

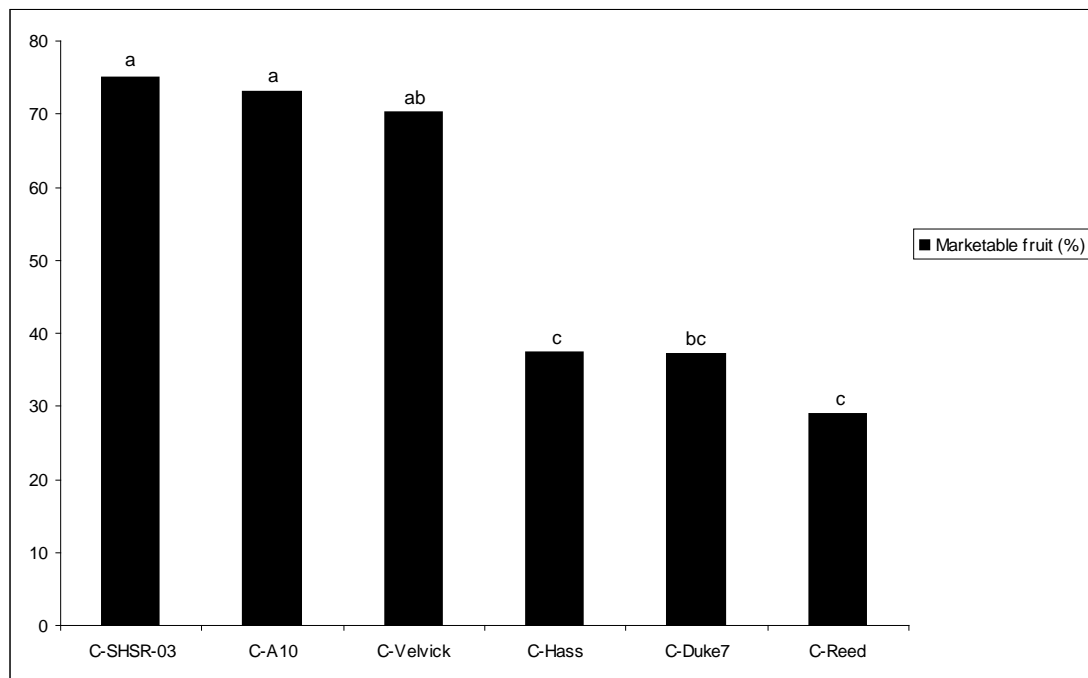
## Results

### *Childers QLD (Hass)*

Levels of anthracnose and stem end rot were very low in fruit harvested from Childers in 2010, and as a consequence, fruit marketability was generally high and there were no significant differences between any of the rootstocks (data not shown).

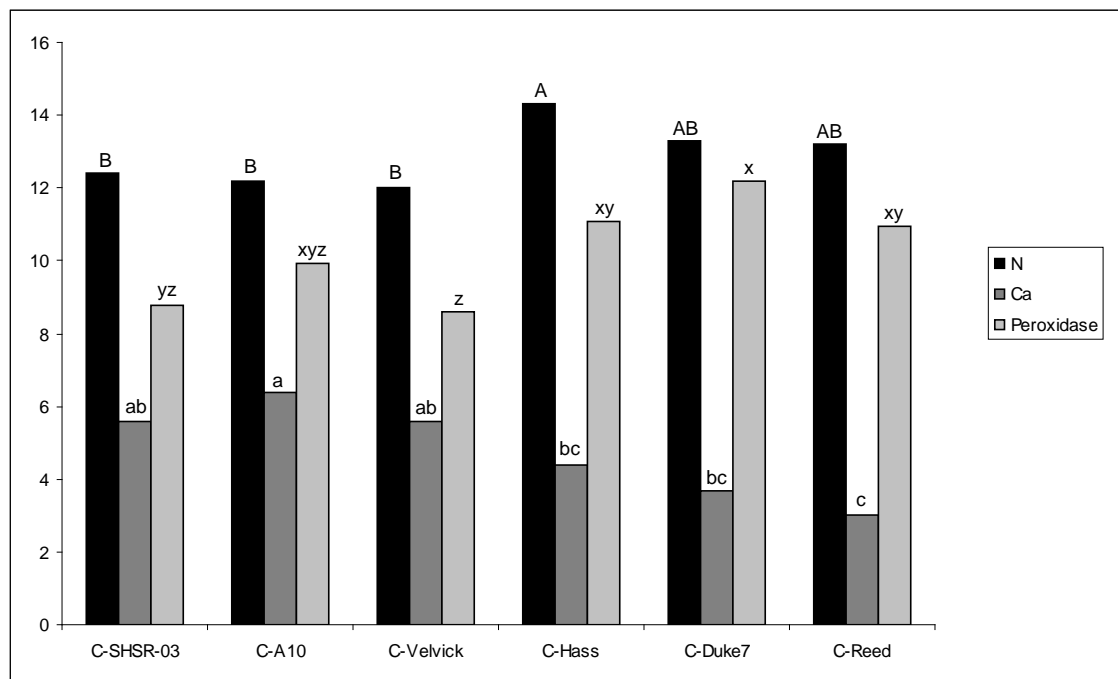
### Hampton QLD (Hass)

At the Hampton site there were some very marked rootstock effects on fruit marketability. Fruit from trees on clonal 'Duke 7', 'Reed' and 'Hass' had very low fruit marketability (due to high anthracnose levels) compared to fruit from trees on clonal 'SHSR-03', 'A10' and 'Velvick' (Fig 1). Similar results were obtained in the previous two seasons (2008 and 2009) at the Hampton field site (data not shown).



**Figure 1: Hampton 2010 - Effects of clonal (C) rootstocks on % marketable fruit in 'Hass' avocado fruit ripened at 23°C. Marketable fruit = % of ripe fruit with a severity rating of 5% or less for anthracnose and no stem end rot. Columns surmounted by the same letter are not significantly different at  $P = 0.05$ .**

The highest nitrogen and lowest calcium levels were seen in fruit which also had the lowest fruit marketability i.e. those from trees on clonal 'Reed', 'Duke 7' and 'Hass' (Fig 2). While there were no significant effects of rootstock on flesh catalase at the Hampton site in 2010 (data not shown), flesh peroxidase levels were significantly lower in fruit from trees on clonal 'Velvick' than in fruit from trees on clonal 'Duke 7', 'Hass' and 'Reed' (Fig 2).

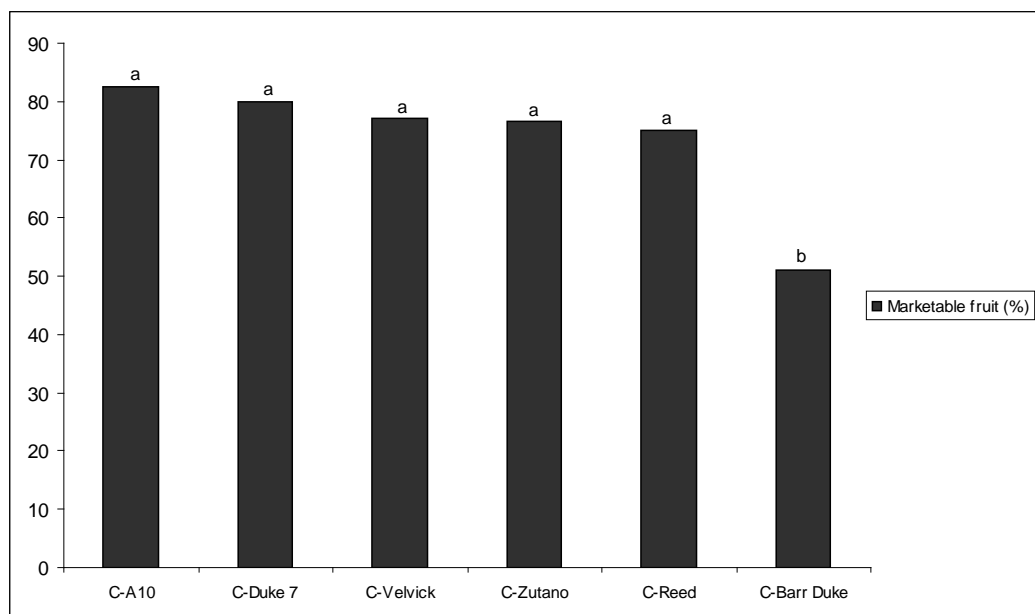


**Figure 2: Hampton 2010 - Effects of clonal (C) rootstocks on fruit skin nutrients (nitrogen and calcium) and fruit flesh peroxidase in 'Hass' avocado. Columns surmounted by the same letter are not significantly different at  $P = 0.05$ . Rootstocks are graphed from left to right in descending order of % marketability.**

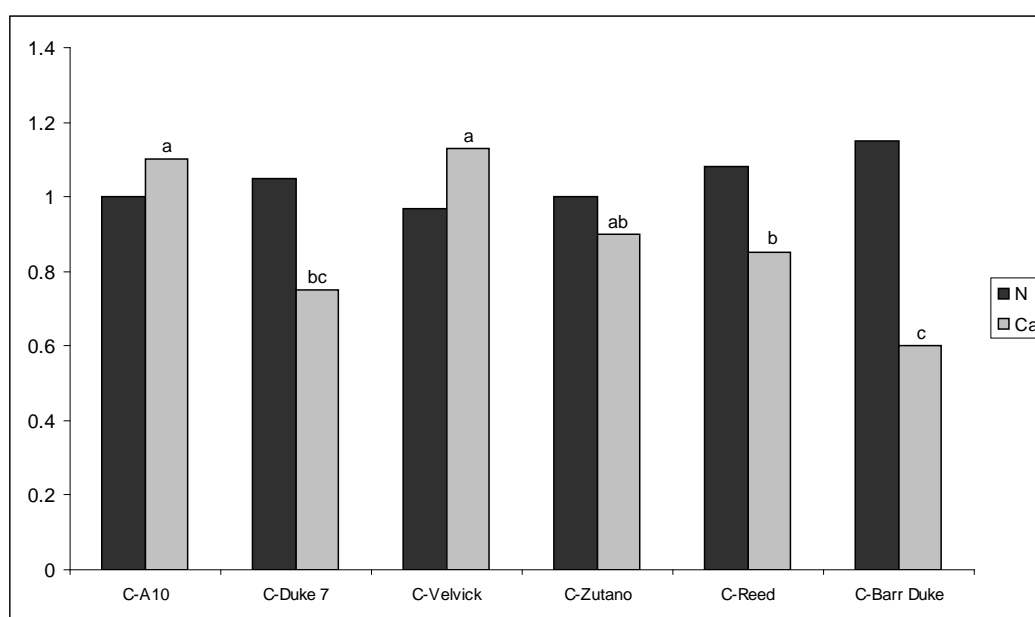
#### *Pemberton WA (Hass)*

Fruit from trees on clonal 'Barr Duke' had a significantly lower marketability percentage (due to high levels of anthracnose) than those from trees on other rootstocks (Fig 3). These fruit also had the lowest skin Ca concentration of all rootstocks, and the highest skin N concentration, although the latter was not significantly different from the other rootstocks (Fig 4). Fruit from trees on clonal 'Velvick' and clonal 'A10' had the highest skin Ca concentrations.

At the Pemberton site there were no significant rootstock effects on fruit defence enzymes (catalase and peroxidase) in 'Hass' fruit (data not shown).



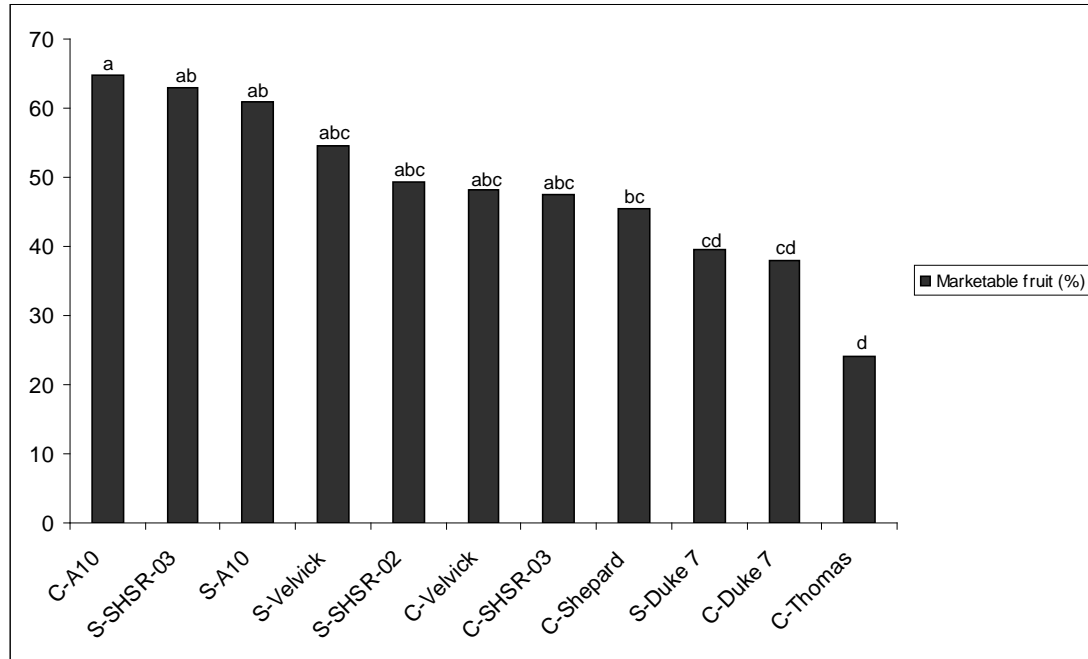
**Figure 3: Pemberton 2010 - Effects of clonal (C) rootstocks on % marketable fruit in 'Hass' avocado fruit ripened at 23°C. Marketable fruit = % of ripe fruit with a severity rating of 5% or less for anthracnose and no stem end rot. Columns surmounted by the same letter are not significantly different at  $P = 0.05$ .**



**Figure 4: Pemberton 2010 - Effects of clonal (C) rootstocks on fruit skin nutrients (nitrogen and calcium) in 'Hass' avocado. Columns surmounted by the same letter are not significantly different at  $P = 0.05$ . Rootstocks are graphed from left to right in descending order of % marketability.**

#### Walkamin QLD (Shepard)

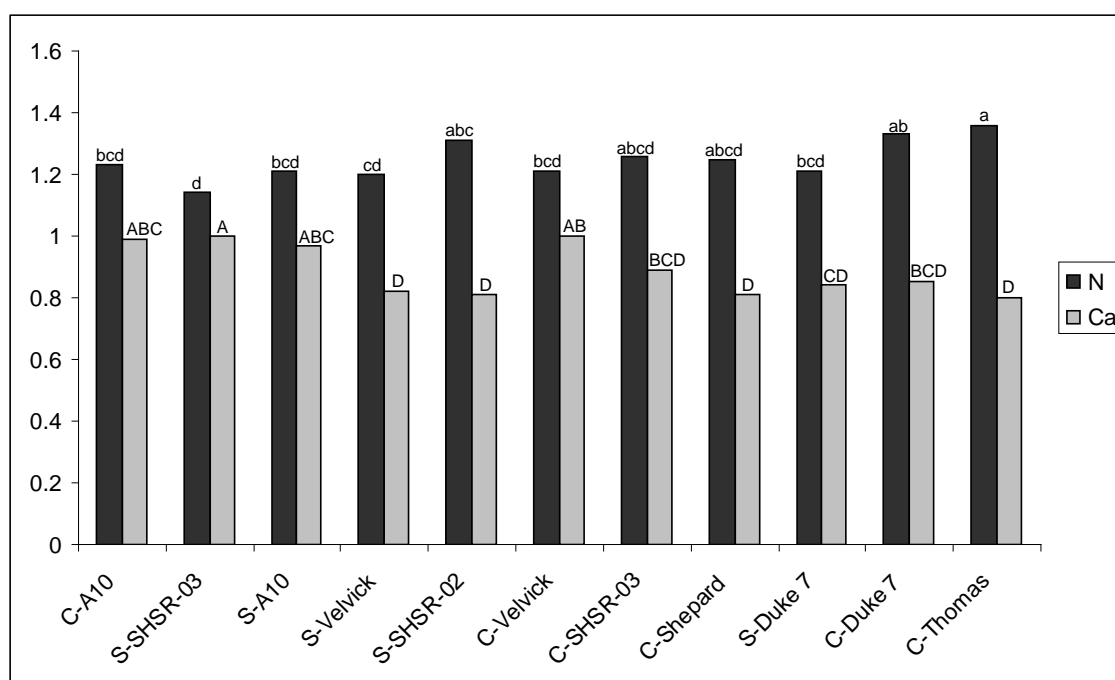
Fruit harvested from 'Shepard' trees grafted to clonal 'Thomas', clonal 'Duke 7' and seedling 'Duke 7' rootstock were the least marketable fruit in this trial due to high levels of both anthracnose and stem end rot, whereas those from clonal 'A10', seedling 'A10' and seedling 'SHSR-03' rootstocks were the most marketable (Fig 5).



**Figure 5: Walkamin 2011 - Effects of clonal (C) and seedling (S) rootstocks on % marketable fruit in 'Shepard' avocado fruit ripened at 23°C. Marketable fruit = % of ripe fruit with a severity rating of 5% or less for anthracnose and no stem end rot. Columns surmounted by the same letter are not significantly different at  $P = 0.05$ .**

Fruit from trees on clonal 'Thomas' had the highest skin N and lowest skin Ca of all treatments (Fig 6), which correlates well with fruit marketability data.

There were no significant effects of rootstock on fruit defence enzymes (catalase and peroxidase) in 'Shepard' fruit at the Walkamin site in 2011 (data not shown).



**Figure 6: Walkamin 2011 - Effects of clonal (C) and seedling (S) rootstocks on fruit skin nutrients (nitrogen and calcium) in 'Shepard' avocado. Columns surmounted by the same letter are not significantly different at  $P = 0.05$ . Rootstocks are graphed from left to right in descending order of % marketability.**

## Conclusions

The results presented in this paper represent the third season of postharvest disease assessments conducted under the project on 'Rootstock Improvement for the Australian Avocado Industry – Phase 3'. While there is variation between sites, propagation method, scion cultivar and season, some trends are starting to emerge. Rootstocks which frequently perform well in terms of reduced postharvest disease levels after ripening at 23°C include 'A10', 'SHSR-03' and 'Velvick'. Conversely, those which tend to be associated with higher postharvest disease levels include 'Barr Duke', 'Thomas', and 'Hass'. There are also some rootstocks which appear to perform better at particular locations. For example, while 'Reed' has not performed so well at the Hampton site in Queensland over a number of years in terms of postharvest disease, it has been a reasonable performer at the Pemberton site in Western Australia.

We continue to see good correlations between fruit nutrient levels and postharvest disease, confirming that rootstock/scion combinations with high fruit N and low fruit Ca levels tend to have higher levels of postharvest disease than those with low fruit N and high fruit Ca levels.

The biochemical markers, catalase and peroxidase, have not to date been strong indicators of fruit disease resistance like fruit N and Ca have. During the 2010/11 season we did observe a correlation between fruit flesh peroxidase levels and fruit disease/marketability at the Hampton site – i.e. fruit with lower peroxidase levels also had lower disease. This result was not seen at the other three field sites however. Further work is currently underway to evaluate other biochemical markers in avocado fruit skin and flesh.



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